UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/39
14 September 1982

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE[1/]

No. 39

CULTIVATION OF PAPAVER SOMNIFERUM AND PAPAVER BRACTEATUM AND THE QUANTITATIVE DETERMINATION OF SOME ALKALOIDS BY HPLC.

by: K. Wickström, H. Pyysalo and C-J. Widèn
Department of Pharmacy
University of Kuopio
Kuopio, Finland

C.A. Salemink
Organic Chemistry Laboratory
State University
Utrecht, The Netherlands

1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

V.82-30348

INTRODUCTION

Within the scope of our work on the isolation of the major and minor alkaloids of Papaver somniferum L. and Papaver bracteatum Lindl., cultivated under different conditions, it was observed that, as a result of certain spray experiments in the Netherlands, (H.G. Theuns 2/) the amounts of some of the main alkaloids were distinctly increased. However, information was lacking on the amounts of the minor alkaloids, many of which were still unknown at the time of the above-mentioned experiments.

In combination with the research on the isolation and structure elucidation of the minor alkaloids, it was decided to cultivate both Papaver species in different regions in Finland, in order to investigate the possible effects on the alkaloid biosynthesis that might be caused by the accelerated and abundant growth during the short spring and summer period in this northern country.

EXPERIMENTAL

Cultivation:

P. somniferum. The seeds (UNL. nos. 101, 106, 107, 110, 119, 136, 154, 187, 275, 316, 364, 593, 3/) were cultivated in the botanical garden of the University of Kuopio (400 km north of Helsinki), where the soil is clayish and also contains a small amount of peat. Each cultivation consisted of 50 to 100 plants in rows at intervals of 25 cm.

Smaller cultivations were also established in the city area of Helsinki and in the village of Lempäälä, 200 km north of Helsinki. These cultivations which consisted of 10 to 20 plants from each batch of seed were located in normal household gardens with mouldy earth. In Helsinki the plants suffered seriously because of the shade of tall trees.

2/ The results will be published in the forthcoming Ph.D. thesis at the State University of Utrecht, The Netherlands.

3/ The seeds of P. somniferum and P. bracteatum were provided through the courtesy of the UN Narcotics Laboratory.

The seeding time of all the cultivations was 20 May 1979. The first seedlings appeared after two weeks, but the growth of the plants was very slow during the month of June. At the beginning of July a highly intensive growth season began and most plants were in bloom during the last two weeks of July. During the month of July the weather conditions in the Kuopio area were excellent; the weather was sunny, the temperature was on average + 20°C, and adequate rain made the cultivations flourish.

In southern Finland the weather conditions were considerably less favourable and the plants were smaller. In Helsinki the plants reached, on average, one third of the size of the plants in Kuopio.

P. bracteatum. The seeds (UNL. nos. 8, 11, 441, 581 and 594) were cultivated in the botanical garden of the University of Kuopio. The growth during the first year was very satisfactory and healthy plants were obtained. Here too, the seeding took place on 20 May 1979 (for experimental conditions, see those used for Papaver somniferum). About 50 % of the plants survived the hard winter (minimum temperature - 36°C) and, at the end of June, some of the second year plants were flowering. The quantitative determination of the alkaloidal content of the leaves of the first year plants indicated the presence of thebaine and alpinigenine as shown in Table 2.

Sampling:

The plants are described in Table 1. Samples were collected during the growing season at intervals of two weeks. Each time, two plants representing the average stage of development in the cultivation were collected (see Table 1). The plants, including the roots, were rinsed with water. Capsule samples, consisting of five average capsules, were collected twice from each cultivation. Immediately after collection, the samples were dried at + 105°C for two hours and stored at room temperature for 2 to 6 months.

After removal of the seeds, the capsule samples were pulverized (diameter of the particles less than 0.5 mm) with the aid of a Culatti mill. The residual moisture in the material was determined by heating an aliquot just above 100°C until constant weight and the average found (8 %) was taken into account in calculating the alkaloid contents.

500 mg of the powdered sample was suspended in 25 ml of acetic acid (5 %) for four hours. The suspension was filtered and the filtrate was extracted with 20 ml of hexane in order to remove pigments etc. The aqueous phase was made alkaline (pH=9) with the aid of ammonia (25 %) and then extracted three times with 20 ml of a chloroform-isopropanol mixture (3:1). The solvent was evaporated to dryness in vacuo and the residue was dissolved in 0.5 ml of methanol.

HPLC experiments:

The methanol solution (10 µl) was injected into a Waters M 6000 HPLC instrument with a 300 mm x 4 mm Bondapak C_{18} column. The absorption detector was a Waters model 400 equipped with a filter at 280 nm (for all the alkaloids) and at 313 nm (especially for thebaine, noscapine and papaverine).

Several experiments were carried out with various solvent systems and the most suitable system in reverse phase chromatography for separating the alkaloids studied was found to be methanol and water (45 . 55) containing 0.05 M of B5-buffer (1-pentanesulfonic acid sodium salt).

The flow rate was 2.0 ml per minute and the pressure 2000 p.s.i.

The quantitative determinations were based on comparison of the peak areas obtained for the samples with those obtained for the reference substances. A HPLC chromatogram for the standard mixture is presented in figure 1.

DISCUSSION

As shown in Table 1, the intensive growth season in the north produces within a short period of time P. somniferum plants containing considerable amounts of the alkaloids studied. Reference data for earlier cultivations from the same batches of seed were available only for samples UNL 136, 316 and 364.

It seems that in certain plants (UNL 316 and 364) the growing conditions in the north cause the plants to produce even higher amounts of the main alkaloids than in the countries of origin. However, lower concentrations were found as well (UNL 136). Seeds from the same batches, cultivated in different parts of the country, produced varying amounts of the main alkaloids, presumably because of the different growing conditions. The poor growth of the plants in Helsinki produced lower amounts of the main alkaloids. The content of the alkaloids analyzed seems to reach a maximum level at the beginning of August. In this stage of the work, only preliminary first analyses for P. bracteatum were made (Table 3). It is evident that P. bracteatum can be cultivated in southern Finland. However, in the Kuopio area, being only a few hundred kilometers south of the Arctic circle,the hard winter with longer periods of time when the temperature is lower than -20°C, a great proportion of the plants could not survive their first winter.

It is interesting to note (table 3) that the concentration of alpinigenine in one of the plants (UNL 581) almost reaches that of thebaine, the main alkaloid in all of the P. bracteatum samples studied. The cultivation will be continued.

Figure 1. A HPLC chromatogram obtained from a mixture of morphine (1),codeine (2),thebaine (3), noscapine (4) and papaverine (5).

TABLE 1. (data from Kucpio)

UNL number	average height of flowering plant cm	average weight of fresh capsules g	colour of flowers
101	75	9.5	white
106	70	7.5	violet
107	100	12.5	white/violet
110	100	18.0	white
119	50	5.5	pink
136	60	3.7	red/violet
154	30	3.5	white
187	40	3.0	white
275	65	3.5	white
316	30	2.6	white
364	90	2.9	white/violet
593	60	5.8	white/violet

TABLE 2. Alkaloid contents of Papaver somniferum samples

Place of growth: K = Kuopio, H = Helsinki, L = Lempäälä

The data given is the alkaloidal content of the whole plants, including roots, except as indicated in certain cases where the data concerns the capsules only.

UNL data = data on capsules from earlier cultivations of the same batch of seed in the country of origin.

Sample	Date 1979	Place	morphine	codeine	thebaine	noscapine	papaverine
UNL 119	13.7.	K	0.040%	0.005%	0.0028%	0.0065%	0.0040%
"Nauga"	31.7.	K	0.170	0.007	0.0130	0.0087	0.0010
Hungary	26.8.	K	0.099	0.003	0.0036	0.0067	<0.0005
	13.8.	L	0.032	-	<0.0005	0.0045	0.0006
	10.9.	L	0.042	-	<0.0005	0.0045	<0.0005
	29.6.	H	0.020	0.0017	0.0015	0.0007	<0.0005
	25.7.	H	0.037	0.0027	<0.0005	0.0030	0.0010
	29.8.	H	0.060	0.0036	0.0008	0.0062	<0.0005
UNL 107	13.7.	K	0.024	0.0020	0.0008	0.0015	<0.0005
Kék Duna	31.7.	K	0.046	0.0055	0.0090	0.0110	0.0020
Hungary	12.8.	K	0.230	0.0070	0.0060	0.0170	0.0009
	26.8.	K	0.160	0.0190	0.0120	<0.0005	<0.0005
	1.9 (Capsules)	K	0.210	<0.0005	0.0012	0.0120	<0.0005
	13.8.	L	0.025	-	<0.0005	0.0010	<0.0005
	10.9.	L	0.042	-	<0.0005	0.0010	<0.0005
	25.7.	H	0.037	0.0027	<0.0005	0.0030	0.0010
	29.8.	H	0.043	0.0023	<0.0005	0.0042	<0.0005

UNL 136	13.7.	K	0.041	0.0280	0.0055	0.0150	0.0100
Pakistan	31.7.	K	0.060	0.0140	0.0060	0.0290	0.0230
	12.8	K	0.300	0.0200	0.0018	0.1600	0.0300
	12.8. (Capsules)	K	0.380	0.0250	0.0060	0.1400	0.1000
	26.8.	K	0.150	0.0210	0.0025	0.0390	0.0550
	1.9. (Capsules)	K	0.340	0.0190	<0.0005	0.1200	0.0940
		UNL data	1.00	0.09	0.07		
UNL 154	13.7.	K	0.057	0.0100	0.0050	0.0450	0.0095
Afghanistan	31.7.	K	0.063	0.0095	0.0082	0.0210	0.0120
	26.8.	K	0.180	0.0260	0.0200	0.0780	0.0230
	13.8.	L	0.085	0.0130	0.0022	0.0400	0.0105
	10.9.	L	0.048	0.0083	0.0011	0.0400	0.0105
UNL 187	13.7.	K	0.031	0.0140	0.0030	0.0080	<0.0005
Jhalawar	31.7	K	0.061	0.0120	0.0045	0.0180	0.0007
Rajasthan	12.8.	K	0.310	0.0230	0.0140	0.1000	<0.0005
India	26.8.	K	0.220	0.0250	0.0090	0.0730	<0.0005
	12.8. (Capsules)	K	0.390	0.0310	0.0080	0.1600	0.0030
	1.9. (Capsules)	K	0.420	0.0092	0.0005	0.1200	0.0015
	10.9.	L	0.170	0.0190	0.0040	0.1200	0.0145

UNL 101	13.7.		K	0.0240	0.0120	0.0028	0.0054	<0.0005
Turkey	31.7.		K	0.0320	0.0070	0.0040	0.0033	0.0025
	12.8.		K	0.130	0.0210	0.0042	0.0600	0.0100
	26.8.		K	0.120	0.0230	0.0092	0.0140	0.0150
	12.8.	(Capsules)	K	0.330	0.0280	0.0050	0.0650	0.0160
	1.9.	(Capsules)	K	0.200	0.0050	0.0014	0.0180	0.0027
	13.8.		L	0.0530	0.0062	0.0035	-	-.0025
	10.9.		L	0.0230	0.0091	0.0005	0.0110	0.0010
	20.6.		H	0.0150	0.0024	0.0005	0.0043	<0.0005
	29.8.		H	0.028	0.0100	0.0005	0.0160	0.0065
UNL 106	13.7.		K	0.070	0.0130	0.0022	0.0030	<0.0005
Turkey	12.8.		K	0.083	0.0063	0.0015	0.0100	<0.0005
	26.8.		K	0.110	0.0066	0.0025	0.0100	0.0063
	13.8.		L	0.069	0.0030	0.0030	0.0120	0.0007
	10.9.		L	0.029	0.0057	0.0013	0.0110	0.0034
	20.6.		H	0.009	0.0033	0.0011	0.0036	<0.0005
UNL 110	13.7.		K	0.073	0.0120	0.0020	0.0012	0.0007
Australia	12.8.		K	0.530	-	-.0060	0.0280	0.0047
	1.9.	(Capsules)	K	0.430	<0.0005	0.0130	0.0390	<0.0005
	10.9.		L	0.022	0.0013	0.0040	0.0110	<0.0005
	25.7.		H	0.025	0.0013	<0.0005	0.0058	<0.0005
	29.8.		H	0.076	0.0050	0.0011	0.0360	<0.0005

UNL 275	13.7.	K	0.040	0.0060	0.0010	0.0100	0.0006
Aklera	31.7.	K	0.039	0.0088	0.0056	0.0086	0.0015
Rajasthan	31.7.	K	0.067	0.0150	0.0067	0.0130	0.0066
India	12.8.	K	0.300	0.0200	0.0066	0.0760	<0.0005
	26.8.	K	0.230	0.0260	0.0190	0.0870	<0.0005
	12.8. (Capsules)	K	0.350	0.200	0.0080	0.1100	<0.0005
	1.9. (Capsules)	K	0.340	0.0290	0.0030	0.1200	0.0028
	13.8.	L	0.071	0.0077	0.0026	0.0630	0.0008
	10.9.	L	0.079	0.0063	0.0011	0.0460	<0.0005
	20.6.	H	0.016	0.0041	0.0027	0.0019	<0.0005
	25.7.	H	0.058	-	-.0022	0.0190	<0.0005
UNL 316	13.7.	K	0.081	0.0120	0.0038	0.0170	0.0025
Faizabad	31.7.	K	0.120	0.0390	0.0070	0.0230	<0.0005
Uttar	12.8.	K	0.270	0.0270	0.0140	0.0920	<0.0005
Pradesh	26.8.	K	0.250	0.0190	0.0140	0.0850	<0.0005
India		UNL data	0.05	0.09	0.05		
UNL 364	13.7.	K	0.044	0.0180	0.0045	0.0160	0.0010
Rustica 0	31.7.	K	0.120	0.0230	0.0065	0.0210	<0.0005
Spain	12.8.	K	0.300	0.0220	0.0035	0.0820	<0.0005
	26.8.	K	0.240	0.0087	0.0240	0.0500	<0.0005
	1.9. (Capsules)	K	0.480	0.0005	0.0024	0.1300	<0.0005
		UNL data	0.34	0.08	-		

UNL 593	13.7.	K	0.150	0.0240	0.0055	0.0020	0.0135
Australia	31.7.	K	0.076	0.0140	0.0280	0.0015	0.0010
	12.8.	K	0.300	0.0220	0.0035	0.0820	<0.0005
	26.8.	K	0.240	-	0.0350	<0.0005	0.0250
	12.8. (Capsules)	K	0.700	0.0120	0.0260	0.0080	0.0020
	1.9. (Capsules)	K	0.330	0.0220	<0.0005	0.1200	0.0980

TABLE 3. Thebaine and alpinigenine content (% in dry material) in *Papaver bracteatum* plants from the 1st year, grown in Kuopio.

Plants	date	thebaine	alpinigenine
UNL 8	21.7.	0.223	<0.005
	26.8.	0.065	<0.005
UNL 11	26.8.	0.113	<0.005
UNL 594	26.8.	0.210	<0.005
UNL 441	21.7.	0.139	<0.005
	26.8.	0.058	<0.005
UNL 581	21.7.	0.080	0.072
	26.8.	0.138	0.086